

# Chemistry and Biology of the Polyene Macrolide Antibiotics

J. M. T. HAMILTON-MILLER

*Department of Medical Microbiology, Royal Free Hospital, Gray's Inn Road, London, W.C.1., England*

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## INTRODUCTION

The polyene group of antibiotics exists as a chemical and biological subdivision of the macrolide class. Antibiotics in this class are characterized by the possession of a macrocyclic ring of carbon atoms closed by lactonization; the polyene group has, in addition, a series of conjugated double bonds. It is the latter which accounts for the biological differences observed between the polyene and erythromycin groups (see Table 1).

There have been several excellent reviews of the polyene group from chemical, biological, and medical points of view (87, 88, 90, 155, 156, 195, 204, 296, 303, 310); however, since the most recent of these appeared there has been a spate of reports in the chemical literature concerning the elucidation of the structures of various polyene antibiotics. Knowledge of the structures of these compounds now permits a much closer reassessment of their interrelationships, properties, and functions than was possible heretofore. The importance of knowing the

structures of antibiotics is considerable; such knowledge will enable studies on the detailed modes of action to advance in a more logical manner, and chemical modifications of the substances can be attempted rationally, with the aim of increasing the pharmaceutical acceptability of the drug, and perhaps of modifying its activity, either by broadening its range or by designing compounds effective against resistant variants of usually sensitive species.

Resistance to the polyenes does not seem to have occurred yet (see below), and with the development of chemically unrelated antimycotic agents such as 5-fluorocytosine and clotrimazole, the problem of resistance may seem unimportant at present, but antibiotic history should have taught medical scientists that eternal vigilance is the watchword. It may be possible to modify a polyene in order to circumvent a specific mode of resistance, to reduce toxicity, or to produce a more acceptable form of the drugs for clinical use. With these problems in mind the present review was compiled.

TABLE 1. *Biological differences between the erythromycin and polyene groups*

Property	Erythromycin group	Polyene group
Antimicrobial spectrum	Active against gram-positive bacteria, <i>Haemophilus</i> , <i>Brucella</i> , and <i>Neisseria</i>	Virtually inactive against bacteria Active against yeasts, molds, and filamentous fungi
Site of action	70S ribosome	Sterol in cell membrane
Mode of action	Inhibits peptidyl synthetase and translocation	Alters cell permeability

## CHEMICAL ASPECTS

### General

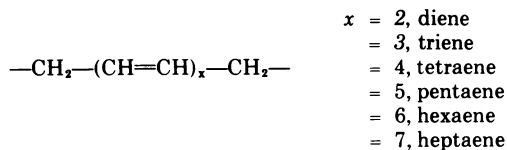
The main reason for the recent burst of success in the extremely difficult field of solving the structures of such complex macromolecules as the polyenes has been the application of the highly sensitive analytical tool of mass spectrometry to the problem. This technique provides, among other useful information, precise data on the molecular weights of components of antibiotic mixtures by examining suitably prepared volatile compounds such as trimethylsilyl or polyacetate derivatives (110, 209). Molecular-weight determination proved to be a major stumbling block in early structural investigations (see e.g. 71, 83, 111). Other physical methods, especially proton magnetic resonance, have greatly helped these studies. However, there is still much dependence upon classical chemical degradative procedures such as periodate oxidation and ozonolysis (62), and similar, newer techniques have been specifically designed for the problem, such as the method devised by Cope et al. (71) for determining the carbon skeleton and the high-pressure hydrogenation procedure of Ceder et al. (63) for investigating the macrolidic moiety of the molecule. The logical sequence of procedures involved in working out the structure of a polyene is well illustrated in the papers by Pandey et al. (207) and Rinehart et al. (229) on the structures of tetrins A and B.

The feature which permits rapid classification of the polyene antibiotics, even in a crude state in culture supernatant fractions, is the highly characteristic nature of their ultraviolet (UV) absorption spectra, which led Oroshnik et al. (205) to suggest that this group of unsaturated compounds was in fact polyenic (i.e. containing only double bonds of the ethylenic type), as opposed to polyenylic (containing a mixture of double and triple bonds). Absorption spectra of polyene compounds have been rationalized by reference to the spectra of model hydrocarbons of known structure containing 4, 5, 6, or 7 conjugated double bonds (204). The

model compounds for trienes have been described by Walker and Hawkins (313).

Fully saturated organic compounds contain only tightly bound electrons and thus absorb UV light of only very short wavelengths. The presence of unsaturation in a molecule, such as a double bond, involving as it does relatively loosely bound electrons, causes absorption to occur at longer wavelengths. Conjugation of double bonds increases this bathochromic effect of unsaturation and is additive. If absorption maxima are shifted enough, the compounds involved will be visibly colored; this process can be seen by inspecting samples of the various polyenes. Trienes are colorless or very pale yellow; tetranes, such as nystatin, Rimocidin, and pimarinin, are pale yellow; pentanes, such as Filipin, are yellow; and heptanes like amphotericin B are definitely orange.

The UV absorption spectrum of a polyene antibiotic usually enables it to be classified at once not only as a polyene, but also more specifically as a triene, tetraene, a subdivision of the pentanes, hexane, or heptane (see I). Se-



#### I. *General formula for conjugated polyene systems, and nomenclature.*

matically, the prefix "poly" referring to a maximum number of seven is probably incorrect, and the purist would prefer the term "oligone" to describe this series of compounds. However, the latter term is both ugly and unfamiliar, so the former will continue to be used. Figure 1 shows graphically the positions of the first three peaks in the absorption spectra of selected trienes, tetranes, pentanes, hexanes, and heptanes, together with the peak attributable to a conjugated diene. It is clear that the illustrated trends are smooth and progressive. A most important inference, from a structural point of view, arising from comparisons of the spectra of polyene antibiotics is that it is

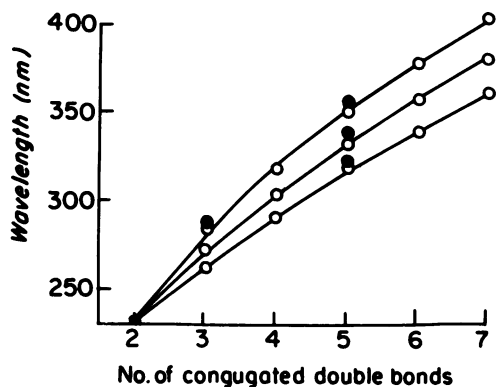
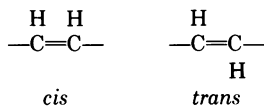


FIG. 1. Relationship between absorption maxima of polyenes and number of conjugated double bonds. The positions of the first three peaks are shown; for compounds with five conjugated double bonds, the filled points are the methylpentanes and the unfilled are the classical pentanes. All figures were corrected to 95% ethanol, wherever possible. Data calculated from references 5, 13, 72, 204, 301, 312.

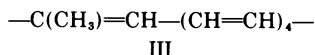
reasonable to assume that, in all the tetrane, pentane, and hexane compounds of this class for which spectra have been recorded, the stereochemistry of the double bonds is all *trans* (II). This conclusion is also valid for the heptanes candidin and amphotericin B (204).

The fine structure and the precise location of

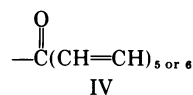


## II. *Cis* and *trans* (geometric) isomerism

the spectral bands of the polyenic systems described above are sensitive to changes in their atomic environment, and advantage is taken of this fact to define a subgroup of the pentanes, the methylpentane group, having a chromophore as in (III). The presence of a methyl group



results in a bathochromic shift of about 6 nm (204). Another example of the effect of neighboring groups altering the characteristics of the polyene spectrum is shown by conjugation with a ketone group, as in the pentanes flavofungin (35) and flavomycoin (242, 243) and the hexane dermostatin (193). In these compounds the usual polyene type of spectrum is absent, and the two absorption peaks in the spectra of the pentanes (at 262 and 363 nm) and the single peak in that of the hexane (at 385 nm) are at longer wavelengths than would be expected. This is a consequence of the chromophore IV,

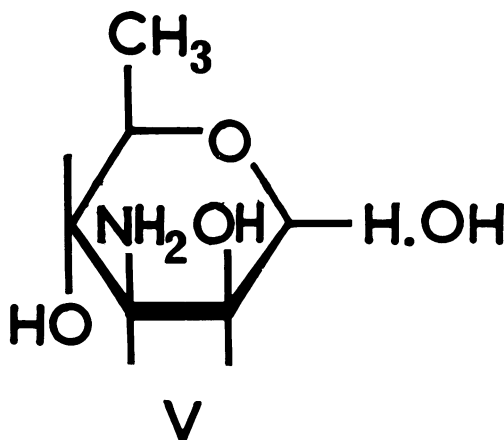


which virtually makes the pentanes into hexanes and the hexane into a heptane. Low-temperature spectra (at  $-173$  to  $-185$  C) of flavomycoin and dermostatin (193) reveal a more typical polyene pattern, but with bathochromic shifts of 32 to 38 and 31 to 41 nm, respectively.

## Classification

The most convenient general way to classify this group is firstly by the number of conjugated double bonds which they possess and secondly by their possession or lack of a glycosidically linked carbohydrate.

The carbohydrate moiety, when present, is, except in one case, the hexosamine mycosamine (3-amino-3,6-dideoxy-D-mannopyranose; V); in



the case of perimycin the sugar is an isomer of mycosamine, perosamine (4-amino-4,6-dideoxy-D-mannose). The structures of these novel carbohydrates were elucidated by Dutcher, Walters, and Wintersteiner (92, 236), Lee and Schaffner (164), and Stevens et al. (364). Mycosamine is closely related to two of the sugars found in the nonpolyene macrolide antibiotics, desosamine and mycaminose (27). The glycosidic bond which connects the carbohydrate to the aglycone has been reported as  $\beta$  in the case of amphotericin B (182), and mycosamine is in the chair conformation. It should be noted that the presence of mycosamine or perosamine automatically confers a basic charge on the molecule, for the pK of the amino group is about 8.6 (86). Many of the polyene antibiotics are amphoteric, possessing one basic and one acidic group; such compounds will be electrophoretically neutral be-

tween pH values of approximately 5 to 9 (e.g., the isoelectric point of pimaricin is 6.5 [221]). The use of the word "neutral" in this sense should not be confused with the same word when it is used to describe a compound like fungichromin which has no ionizable groups. There is little information on pK values for the carboxyl groups present in most of the polyene antibiotics; a value of 4 to 4.5 has been quoted for nystatin (160) and 5.1 for tetramycin (86).

General classification cannot go beyond this; however, in each group additional chemical subdivisions specific to that group can be made, and these will be outlined below. Unfortunately, data on many of the reported polyenes is insufficient for them to be classified completely but, by assuming that those compounds reported to contain nitrogen in fact possess a carbohydrate moiety (unless this is specifically contradicted [72]), we can classify most of the eighty-odd compounds listed in Tables 2 to 9.

Another problem in attempting a classification of the polyenes is that these compounds are often extraordinarily difficult to obtain in pure form, and so data given may not in fact be applicable to the strictly pure compound. There is doubt, for instance, whether the pentane aliomycin (139), reported to contain sulfur, was in fact pure when analyzed. Several polyenes once thought to be pure have subsequently been resolved into separate, closely related but chemically distinct fractions. Thus, Filipin consists of at least eight related compounds (25, 209), nystatin is composed of two fractions, A<sub>1</sub> and A<sub>2</sub> (250), and candidin has recently been resolved into three fractions (41).

The different groups of polyenes will be considered in the following sections.

**Trienes.** The first member of this group, MM-8, was reported as recently as 1965; it is similar to, but not identical with, mycotrienin; neither contains carbohydrate. Trienine is reported to be cytotoxic and is of about twice the molecular weight of mycotrienin. MM-8 has been calculated to have a molecular weight of 726, which implies two atoms of nitrogen per molecule, as it is stated to contain about 4% N. Resistaphylin and proticin differ from the other polyene macrolides in having great activity against bacteria and little activity against yeasts and fungi; the molar extinction coefficient of the former compound is only about 65% of that of mycotriene and trienine, which suggests a structural difference (possibly conjugation) from the other trienes. Proticin is unique among the polyenes in containing phosphorus (Table 2).

The conjugated triene variotin, produced by *Paecilomyces varioti* var. *antibioticus* (323) is not included here because it is not a macrolide. Takeuchi and Yonehara (271) have shown its structure to be *N*-(8'R-hydroxy-6'-methyl-dodeca 2',4',6'-trienoyl)-2-pyrrolidone; the triene chromophore is conjugated with a ketone group, which accounts for its anomalous absorption spectrum ( $\lambda$  max 318 to 324 nm;  $E_m$  of 34,900) compared with other trienes. It should be noted that the  $E_m$  of variotin is very close to that of resistaphylin.

**Tetranes.** As can be seen from Tables 3 and 4, there is a good deal known about the chemistry of the tetrane group, and full structures (except for stereochemistry) are known for six compounds. There is one subgroup, the epoxide group, represented by pimaricin (Natamycin), lucensomycin (Etrusco-mycin), and PA 166, within the mycosamine-containing group; Rinehart et al. (229) have suggested that lucensomycin and PA 166 are in fact identical. The oxirane ring present in these three compounds is one exception to the general rule that the macrolide antibiotics contain only one ring structure. It is interesting that the nonpolyene macrolides magnamycin and oleandomycin also contain epoxide structures. The tetrins, sistomycosin, protocidin, and endomycin are soluble in water. The endomycins are co-produced with a complex of nonpolyenic compounds with antifungal activity, in which the major components have been called enhygrofungin, U-29,479, and scopafungin. The description of "endomycin" by Gottlieb and Carter (117) can be seen by reference to later work on crystalline scopafungin (26, 140) to refer to the latter compound rather than to the polyene components. The molecular weights of the tetranes range from 666 (pimaricin) to 926 (nystatin); taking the mean observed value of  $E_m$  for tetranes as 80,460, it can be calculated from data on  $E_{1\%}$  that the molecular weights of unamycin A, sistomycosin, and endomycin A are approximately 800, 2,360, and 845, respectively. The 2,360 molecular weight value suggests that the preparation of sistomycosin used for spectral measurements may not have been pure.

Chromin, the tetrane produced by *Streptomyces chromogenes*, should not be confused with kromin, a degradation product of the (nonpolyenic) macrolide picromycin (202).

Polifungin A is identical to nystatin (218, 231), but polifungin B is distinguishable from the latter. It is not clear whether the tetrane produced by *Streptomyces fungicidicus* and described by Umezawa et al. (287) and the

TABLE 2. Some properties of the trienes

Name	Reference		Producing organism	Chemical composition and molecular weight	$E_m$ (or $E_1^{1\%}$ ) %
	Discovery	Chemistry			
MM-8	13	301	<i>Streptomyces</i> sp.	N, 4	(730 at 272)
Mycotrienin	72		<i>Streptomyces</i> sp.	$C_{36}H_{50}N_2O_8$ (638)	54,400 at 272
Proticin	196		<i>Bacillus licheniformis</i> var. <i>mesentericus</i>	$C_{31}H_{45}O_7P$ (560)	28,600 at 272
Resistaphylin	5		<i>S. antibioticus</i>	$C_{24}H_{34}N_2O_7$ (463)	34,700 at 275
Trienine	16		<i>Streptomyces</i> sp.	C, 54.7; H, 8; N, 1.3 to 1 (1,300)	50,700 at 267

TABLE 3. Some properties of mycosamine-containing tetranes

Name	Reference		Producing organism	Chemical composition and molecular weight	$E_m$ (or $E_1^{1\%}$ )
	Discovery	Chemistry			
Amphotericin A	298	109	<i>Streptomyces nodosus</i>	C, 60.3; H, 8.4; N, 1.7 (915)	82,800 at 303
Lucensomycin	9	106-108	<i>S. lucensis</i>	$C_{36}H_{53}NO_{13}$ (708)	98,500 at 305
Nystatin	127	66	<i>S. albulus</i>	$C_{47}H_{75}NO_{17}$ (926)	78,600 at 305
			<i>S. noursei</i>		
PA 166	151	229	<i>Streptomyces</i> sp.	$C_{35}H_{53}NO_{14}$ (712)	78,200 at 304
Pimaricin (Tennecetin)	266	111	<i>S. natalensis</i>	$C_{33}H_{47}NO_{14}$ (666)	74,000 at 303
			<i>S. chattanoogensis</i>		
			<i>S. gilveosporus</i>		
Rimocidin	79	70	<i>S. rimosus</i>	$C_{38}H_{63}NO_{13}$ (742)	71,500 at 304
Tetramycin	86		<i>S. noursei</i> var. <i>jenensis</i>	$C_{34}H_{53}NO_{14}$ (699)	83,900 at 304
Tetrin A	120	207	<i>Streptomyces</i> sp.	$C_{34}H_{51}NO_{13}$ (681)	78,300 at 303
Tetrin B	120	229	<i>Streptomyces</i> sp.	$C_{34}H_{51}NO_{14}$ (697)	78,300 at 303
Unamycin A	179		<i>S. fungicidicus</i>	C, 52.2; H, 7.8; N, 1.7; O, 38.3	(1,010 at 304)

TABLE 4. Some properties of tetranes for which insufficient data exists for classification to be made

Name	Reference		Producing organism	Chemical composition	$E_m$ (or $E_1^{1\%}$ )
	Discovery	Chemistry			
Akitamycin	258	100	<i>Streptomyces akitaensis</i>	C, 53.7; H, 7.7; N, 1.65	
Antimycoin A	226		<i>S. aureus</i>		
Chromin	307	308	<i>S. chromogenes</i>	C, 58.2; H, 7.8; N, 2.3	
Endomycin A	116	305	<i>S. endus</i>		(950 at 303)
			<i>S. hygroscopicus</i> var. <i>enhygrus</i>		
Polifungin	152	218	<i>S. noursei</i> var. <i>polifungini</i>		
Protocidin	232	10	<i>Streptomyces</i> sp.	$C_{29}H_{45}NO_{13}$ (615)	43000 at 303
7071-RP	82		<i>Streptomyces</i> sp.	C, 58.3; H, 8; N, 1.65; O, 31.5 (859)	
Sistomycosin	95		<i>S. viridosporus</i>	Contains N	(340 at 306)

substance called yunamycin (285) are in fact the same as unamycin A (179, 284), but it seems likely.

Fumagillin, a tetranic compound produced by *Aspergillus fumigatus* (94), does not have a macrolide ring so it has not been included in Tables 3 and 4. The complete structure of this

compound has been described (272); its UV absorption spectrum shows maxima at 336 and 351 nm, which is explained by the fact that its tetranic chromophore is conjugated to a ketone group. The  $E_{0.1\%}$  for pure fumagillin is 147.5 at 351 nm (104), which gives a  $E_m$  of 67,300. Of the antibiotics in the tetranic group, it seems

that only Rimocidin definitely lacks a carboxyl group (70); most of the other compounds have been described as amphoteric. Sistomycosin is claimed to be neutral (212).

Tetrahexin is an apparently unique compound which contains both hexane and tetraene chromophores (74).

**Pentanes.** There are three subgroups in this class, the methylpentanes and the "lactone-conjugated" group (Table 5), both of which show anomalous UV absorption spectra and the classical or normal group (Table 6). Methylpentanes have a characteristic feature in their infrared spectra, an absorption band at  $850\text{ cm}^{-1}$  due to the trisubstituted double bond (318), which also helps distinguish them from the other subgroups.

Technically, the name "Filipin" is a trade name (Upjohn), and the approved name of this antibiotic is filimarisin; however, because the former name has been used virtually to the exclusion of the latter, the name Filipin will continue to be used here. The methylpentaene durhamycin should not be confused with du-

ramycin, a polypeptide co-produced with the heptane F-17-C (azacolutin) by *Streptomyces cinnamomeus* var. *azacoluta* (77, 253). Moldicidin A differs from the other methylpentaenes in possessing nitrogen and being amphoteric; the other members of the subgroup do not have any ionizable group. The latter property is also shared by the three members of the "lactone-conjugated" group, mycoticins A and B (which are identical, respectively, with the minor and major components of flavofungin [35, 291]) and flavomycoin. Roseofungin, produced by *Streptomyces roseoflavus* (302), may be identical to flavomycoin. It is interesting that the  $E_m$  for the lactone-conjugated group is considerably lower than those for the other two groups. Among the classical pentanes, aliomycin is unusual in its claimed possession of sulfur and for being water soluble, fungichromatin for having no nitrogen, and capacidin for having significant, but low, antibacterial activity.

The genus *Chainia*, a species of which produces chainin, is closely related to *Streptomyces* (273).

TABLE 5. Some properties of methylpentanes and lactone-conjugated pentanes

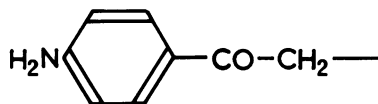
Name	Reference		Producing organism	Chemical composition	$E_m$ (or $E_1$ , 1%)
	Discovery	Chemistry			
<b>Methylpentanes</b>					
No carbohydrate:					
Aurenin	292		<i>Streptomyces aureorectus</i>	$C_{33}H_{54}O_{11}$ (626)	
Cabicidin	200		<i>S. gougeroti</i>	$C_{35}H_{60}O_{13}$ (688)	
Chainin	112	208	<i>Chainia</i> sp.	$C_{33}H_{54}O_{10}$ (610)	80,400 at 338
Durhamycin	115		<i>S. durhamensis</i>	C, 63.8; H, 10.2; O, 25.5	
Filipin complex (I-IV)	318	209	<i>S. filipensis</i>	$C_{38}H_{68}O_{11}$ (655) (Filipin III)	96,400 at 356
Fungichromin (lagosin)	282	71	<i>S. cinnamomeus</i> var. <i>cinnamomeus</i> <i>S. roseoluteus</i> <i>S. cellulosa</i>	$C_{38}H_{68}O_{12}$ (671)	98,800 at 356
Neopentaene	31		<i>Streptomyces</i> sp.	C, 61.3; H, 8.8	(1,380 at 356)
Pentaene	168		<i>S. sanguineus</i>	C, 62.1; H, 9.2; O, 28.9	(1,568 at 338)
Pentamycin (moldicidin B)	290	201, 289	<i>S. pentaticus</i>	$C_{38}H_{60}O_{13}$ (688)	100,500 at 359
Xantholycin	260	247	<i>S. xantholyticus</i>	C, 70.6; H, 9.8; O, 19.7	
<b>Contains carbohydrate:</b>					
Moldicidin A	234	12	<i>Streptomyces</i> sp.	$C_{42}H_{81}NO_{19}$ (903)	76,800 at 339
<b>Lactone-conjugated pentanes</b>					
Flavomycoin	241	242, 243	<i>S. roseoflavus</i> var. <i>jenensis</i>	$C_{41}H_{68}O_{10}$ (721)	62,000 at 363
Mycoticin A (Flavofungin)	53	316 (36)	<i>S. ruber</i>	$C_{37}H_{60}O_{10}$ (664)	61,800 at 364
Mycoticin B			<i>S. flavofungini</i>	$C_{36}H_{58}O_{10}$ (651)	

TABLE 6. Some properties of "classical" pentanes

Name	Reference		Producing organism	Chemical composition and molecular weight	$E_m$ (or $E_1$ %)
	Discov-ery	Chemis-try			
Contain mycosamine: Eurocidin A .....	192	134	<i>Streptomyces albireticuli</i> <i>S. reticuli</i> <i>S. eurocidicus</i>	$C_{40}H_{85}NO_{15}$ (800) $C_{39}H_{83}NO_{15}$ (787)	
Eurocidin B .....					
Contain N in unspecified form:					
Aliomycin .....	139		<i>S. acidomyceticus</i>	Contains S	
Capacidin .....	50		<i>Streptomyces</i> sp.	$C_{54}H_{95}N_2O_{18}$ (1,049)	108,800 at 332 (1,222 at 338)
HA 106 .....	276		<i>Streptoverticillium cinnamoneum</i> var. <i>sparsum</i>		(1,187 at 348)
HA 135 .....			<i>Streptoverticillium sporiferum</i>		(739 at 338)
HA 145 .....			<i>Streptoverticillium cinnamoneum</i> var. <i>albosporum</i>		(1,260 at 338)
HA 176 .....			<i>Streptoverticillium cinnamoneum</i> var. <i>lanosum</i>		
PA 153 .....	151		<i>Streptomyces</i> sp.	$C_{37}H_{61}NO_{14}$ (744)	107,500 at 349
Pentane .....	167		<i>S. effluvis</i>	C, 58.4; H, 8.2; N, 2; O, 21.3	
Compound 616 .....	65		<i>S. parvisporogenes</i>	C, 62; H, 7.8; N, 2.7; O, 27.5	
Insufficient data for further classification:					
Distamycin B .....	257		<i>S. distallicus</i>		(820 at 333)
Fungichromatin .....	282		<i>Streptomyces</i> sp.	No N	(1,550 at 333)

**Hexanes.** Hexanes are the least studied group of polyenes (Table 7). Only dermostatin has received much attention from the structural point of view. It has been shown recently (193) that dermostatin (also known as viridofulvin) has a conjugated hexane-ketone chromophore. Cryptocidin has a very similar  $E_m$  value, and so may have the same sort of chromophore. Flavacid and fradycin are both weakly acidic; it has been suggested (133, 128) that the latter is identical to mycelin. Indeed, Arai and Aiiso (8) reported that mycelin is produced by *Streptomyces fradiae*, whereas this species was claimed to produce fradycin by Swart et al. (268). It is not clear how mycelin and mycelin IMO differ.

**Heptanes.** There are three subgroups of heptanes; mycoheptin, X-63, amphotericin B, and candidin contain no aromatic moiety (Table 8); the candidin group (Table 9) all have a side-chain of *p*-aminoacetophenone (VI); and in the third group this aromatic sidechain is *N*-methylated. Candidin was crystallized and obtained in apparently pure form by Vining and Taber (304),



VI

but it has recently been separated into three fractions, named candidin, candidoin, and candidinin (41).

It is in the group containing *p*-aminoacetophenone that the problem of antibiotic identity shows itself in its most acute form. It is not clear whether candidin, trichomycin, hamycin, ascosin, and levorin are in fact separate entities or are merely mixtures in different proportions of the same basic compounds (54, 84, 136, 312). Kholkhova et al. (143) suggest that *Streptomyces griseus*, *S. canescens*, and *S. levoris* are identical strains, but they found *S. hachijoensis* to be a separate strain. The question of the identity of the antibiotics can be settled either by a detailed chromatography

TABLE 7. Some properties of hexanes

Name	Reference		Producing Organism	Chemical composition and molecular weight	$E_m$ (or $E_1$ %)
	Discovery	Chemistry			
Contains no carbohydrate: Dermostatin	274	193	<i>S. viridogriseus</i>	C, 64.9; H, 9; O, 26.1 (572)	57,200 at 383
Insufficient data for further classification: Cryptocidin	233	11	<i>Streptomyces</i> sp.	$C_{82}H_{88-98}NO_{17}$ (983-986)	57,500 at 382 (1,470 at 380)
Endomycin B	116	305	<i>S. hygroscopicus</i> var. <i>enhygrus</i>		
Flavacid	270	153	<i>S. endus</i> <i>S. flavus</i>	C, 61.6; H, 7.8; N, 1.1; O, 36.3	
Fradicin	268		<i>S. fradiae</i>		
Mediocidin	203	286	<i>S. medicidicus</i>		
Mycelin	4	294	<i>S. roseoflavus</i> <i>S. diastatochromogenes</i> <i>S. fradiae</i> <i>S. diastatochromogenes</i>	no N	(370 at 294)
Mycelin IMO	199		<i>S. diastatochromogenes</i>		
Tetrahexin <sup>a</sup>	75	74	<i>Streptomyces</i> sp. ATCC 14972	C, 65; H, 8.5; N, 1.3; O, 24.7	

<sup>a</sup> Tetrahexin also contains a tetrane chromophore.

TABLE 8. Some properties of heptanes without aromatic moiety, heptanes with *N*-methyl-*p*-aminoacetophenone, and those for which insufficient data has been published

Name	Reference		Producing organism	Chemical composition and molecular weight	$E_m$ (or $E_1$ %)
	Discovery	Chemistry			
Contain no aromatic moiety: Amphotericin B	109	182	<i>Streptomyces nodosus</i>	$C_{47}H_{73}NO_{17}$ (924)	172,000 at 406
Candidin	269	41	<i>S. viridoflavus</i>	$C_{47}H_{71}NO_{17}$ (922)	176,000 at 406
Mycocheptin	42		<i>S. netropsis</i>	$C_{48}H_{77}NO_{17}$ (939)	169,000 at 406
X-63	141		<i>Streptomyces</i> sp.		(700 at 383)
Contain <i>N</i> -methyl- <i>p</i> -aminoacetophenone: Candimycin	251	134	<i>S. echimensis</i>	C, 57.2; N, 1.7; H, 8.2	
DJ400 B <sub>1</sub>	37	38	<i>S. surinam</i>	$C_{66}H_{96}N_2O_{22}$ (1,269)	136,300 at 380
Perimycin	206	165	<i>S. coelicolor</i> var. <i>aminophilus</i>	$C_{47}H_{75}N_2O_{14}$ (892)	89,200 at 383
Insufficient data for further classification: Antifungin 4915	123	154	<i>S. paucisporogenes</i>	C, 63.6; H, 7.8; N, 2.8; O, 24.5	(764 at 380)
Eurotin A	259		<i>S. griseus</i>		
Heptane 757	76		<i>Streptomyces</i> sp.		
Monicamycin	122		<i>Streptoverticillium cinnamomeus</i> var. <i>monicae</i>	C, 58.3; H, 7.85; N, 2.2	
Neoheptane	278		<i>Streptomyces</i> sp.		
PA 150	151		<i>Streptomyces</i> sp.	$C_{54}H_{82}N_2O_{18}$ (1,047)	107,000 at 377



TABLE 9. Some properties of heptanes which contain *p*-aminoacetophenone

Name	Reference		Producing organism	Chemical composition and molecular weight	$E_m$ (or $E_1^{1\%}$ )
	Discovery	Chemistry			
Ascospin	132		<i>S. canescens</i>		
Aureofungin	277	30	<i>S. cinnamomeus</i> var. <i>terricola</i>	C, 60.4; H, 7.9; N, 2.2	
Ayfactin A	142	48	<i>S. viridofaciens</i>	C, 62.6; H, 7.8; N, 2.7	(526 at 383)
Ayfactin B			<i>S. aureofaciens</i>	C, 62.5; H, 7.6; N, 2.8	(556 at 383)
Azacolutin (F-17-C)	220	77	<i>S. cinnamomeus</i> var. <i>azacoluta</i>		
Candididin	161	312	<i>S. griseus</i>	ca. C <sub>83</sub> H <sub>85</sub> N <sub>2</sub> O <sub>19</sub> (1,200)	138,000 at 380
DJ 400 B <sub>2</sub>	37	38	<i>S. surinam</i>	C <sub>58</sub> H <sub>86</sub> N <sub>2</sub> O <sub>21</sub> (1,147)	118,800 at 380
Hamycin	275	81	<i>S. primprina</i>	C, 59.9; H, 7.8; N, 2.2; O, 20.1	(1,060 at 384)
Heptamycin	131		<i>Streptomyces</i> sp.		
Levorin A	43		<i>S. levoris</i>	C <sub>59</sub> H <sub>83</sub> N <sub>2</sub> O <sub>22</sub> (1,181)	118,100 at 379
Levorin B			<i>S. hachijoensis</i>	C <sub>62</sub> H <sub>114</sub> N <sub>2</sub> O <sub>22</sub> (1,238)	123,800 at 381
Trichomycin A	136	126	<i>S. abikoensis</i>	C <sub>61</sub> H <sub>86</sub> N <sub>2</sub> O <sub>21</sub> ·2H <sub>2</sub> O (1,230)	104,400 at 382

study or by mass spectroscopy. Heptamycin, the ayfactins, F-17-C, PA 150, and 757 are probably members of the candididin group. The mixture of ayfactins A and B (so called because they are antiyeast factors) has been called aureofactin and may be the same as the aureofacin described by Igarashi et al. (137); however, aureofungin as described by Thirumalachar et al. (277) is different.

Perimycin has had two previous designations, fungimycin and 1968 Nepera; Borowski et al. (44) at first reported the aromatic moiety to be *p*-aminophenylacetone, but later work (164) showed its true identity to be *N*-methyl-*p*-aminoacetophenone. Perimycin is unusual among the heptanes in having no carboxyl group and unique in possessing perosamine as its carbohydrate moiety.

Amphotericin B and candidin, which have only one atom of nitrogen per molecule, contain a significantly lower percentage of nitrogen (1.5%) than do the other heptanes, which have, by weight, from 2.2 (hamycin and DJ 400 B<sub>1</sub>) to 3.15% (perimycin) of this element. Candimycin, whose reported nitrogen content is 1.7% (251) and which must have at least two atoms of nitrogen per molecule, must therefore have a molecular weight of 1,650, considerably greater than any substantiated, reported molecular weight for polyenes. Antifungin 4915 (2.8%), PA 150 (2.7%), and monicamycin (2.2%) thus seem to fall outside the amphotericin B-candidin group, but there is no evidence published about their possession of an aromatic moiety. *Streptovorticillium cinnamoneus* may be a synonym for *Streptomyces cinnamoneus* (230), so moni-

camycin may in fact be the same substance as F-17-C. From an inspection of values of  $E_m$ , it seems that the group without an aromatic moiety has significantly higher intrinsic UV absorption than do members of the other groups (mean values of  $E_m$  172,330 versus 116,950,  $P < 0.001$ ). On this basis, X-63, antifungin 4915, ayfactin A, and ayfactin B can be calculated to have molecular weights of about 2,500, 1,500, 2,200, and 2,100, respectively.

#### Molar Extinctions of the Polyene Antibiotics

Nayler and Whiting (194) have discussed the numerical values of  $E_m$  for simple polyene series, and they suggested that the simple, empirical relationship  $E_m = n \times 25,000$  (where  $n$  = number of conjugated double bonds) fitted the observed facts. The observed mean values of  $E_m$  for trienes, tetranes, pentanes, and two groups of heptanes are given in Table 10. It is clear that only in the case of the nonaromatic heptanes is the observed value approximately as expected; end effects would be expected to cause deviation from theoretical values in the lower members of the series (194) and, indeed, simple model compounds do not conform precisely to the suggested relationship (180, 261).

It may also very well be that some of the published  $E_m$  values for the polyene antibiotics are low, because of impurity. A closer approximation to a simple empirical relationship is, in fact,  $E_m = (n - 1) \times 25,000$ , at least for the trienes, tetranes, and pentanes. Regression analysis reveals that an alternative expression is  $E_m = n \times 21,000$ .

An interesting observation is that, when the

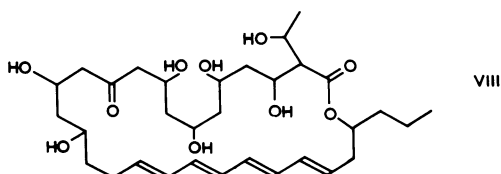
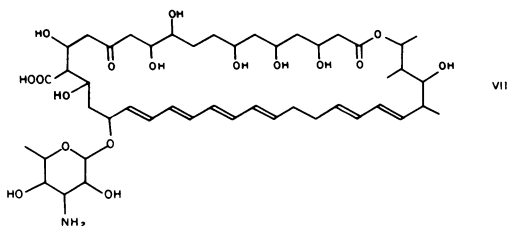
TABLE 10. Mean values and standard deviations of  $E_m$  for different classes of polyene antibiotics

Class of compound	Mean $E_m \pm$ SD (from Tables 2-9)
Trienes	52,550 (2)
Tetraenes	80,460 (9) $\pm$ 7,770
Pentaenes	95,590 (7) $\pm$ 12,510
Heptaenes	
All	132,050 (11) $\pm$ 29,350
Amphotericin B group	172,330 (3) $\pm$ 3,510
Others	116,950 (8) $\pm$ 16,456

chromophoric polyene system is conjugated with the lactone carbonyl, there is a substantial decrease in  $E_m$  (cf. variotin, fumagillin, flavomycolin and the mycoticins, and dermostatin).

### Chemical Structures

Only in the case of amphotericin B (102, 182) is the full structure, with regard to stereochemistry at 14 asymmetric centers as well as the configuration of the glycosidic link, of a polyene known. For 12 other antibiotics (nystatin, pimaricin, lucensomycin, tetrins A and B, chainin, aurenin, fungichromin, Filipin III, mycoticins A and B, and candidin), full structures except for stereochemistry have been proposed, whereas the aglycones of rimocidin and DJ400 B<sub>1</sub> and B<sub>2</sub> have had structures (without stereochemistry) assigned. The structures put forward for all these compounds are given here (VII to XXI) (Table 11).



It is clear at a glance that all the polyenes have in common the possession of clearly demarcated hydrophilic (polyol) and hydrophobic (polyene) regions, structures which help to explain some of their peculiar properties, in particular their detergent-like action (146). The numbers of atoms in the macrocyclic lactone

rings of the polyenes are substantially greater than those found in the nonpolyene group (e.g., 12 in picromycin and methymycin, 14 in erythromycin, 16 in magnamycin, and 17 in spiramycin [300]).

The similarities between the tetrins (XI and XII), pimaricin (IX), and lucensomycin (X) have been remarked upon (229); it is clear that rimocidin (VIII) also resembles these structures, except that it has a 28-membered lactone ring, and it lacks both the isolated 2 to 3 double bond and the epoxide linkage. By analogy with structures IX to XII, one would expect the point of attachment of the mycosamine ring in Rimocidin to be C-17. Nystatin (VII) does not resemble

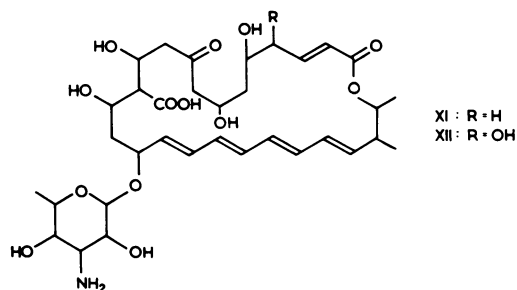
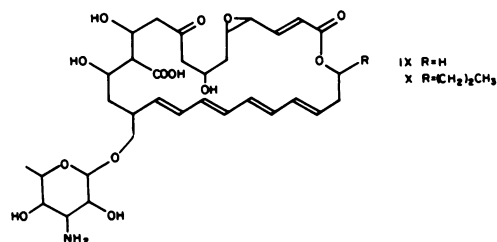
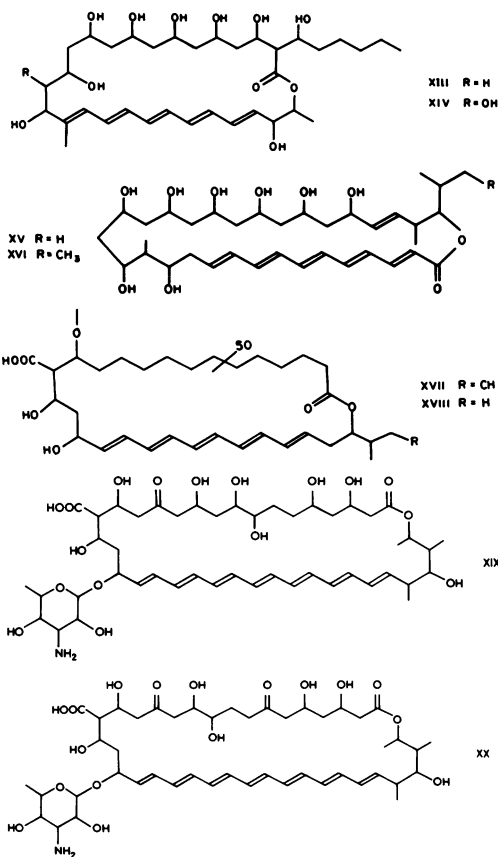


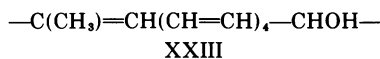
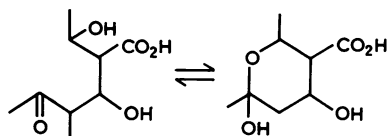
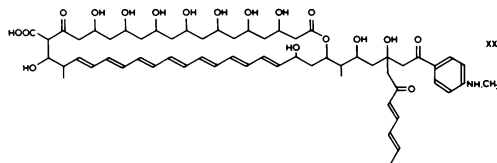
TABLE 11. Polyene antibiotics for which detailed structural proposals have been advanced

Class	Compound	Structure no.	Size of macrolide ring
Tetraenes	Nystatin A	VII	38
	Rimocidinolide	VIII	28
	Pimaricin	IX	26
	Lucensomycin	X	26
	Tetrin A	XI	26
	Tetrin B	XII	26
Pentaenes	Chainin		28
	Filipin	XIII	28
	Fungichromin	XIV	28
	Mycoticin A	XV	32
	Mycoticin B	XVI	32
	Eurocinolide A	XVII	30
Heptaenes	Eurocinolide B	XVIII	30
	Amphotericin B	XIX	38
	Candidin	XX	38
	Aglycone of DJ400B <sub>1</sub>	XXI	38



fungichromin. The structure proposed by Pandey et al. (208) for chainin is closely related to that of Filipin III (XIII), chainin being the latter with a slightly modified C-2 sidechain (*n*-butyl in place of  $\alpha$ -hydroxy-*n*-hexyl). The structure of aurenin proposed by Ushakova et al. (292) may require revision, for the size of the lactone ring (24 members) is smaller than that found among the other pentanes.

*Streptomyces ruber* produces mycoticins A and B in about equal proportions (316), whereas flavofungin consists of about 90% mycoticin B and only 10% mycoticin A (35). Schlegel and Thrum (242) have elucidated the structure of the chromophore of flavomycoïn, which is the same as in mycoticin; flavomycoïn is a larger molecule and also possesses an extra branch in



the other tetranes, but instead has a very close relationship to amphotericin B (XIX) and candidin (XX), and only its lack of a 28 to 29 double bond differentiates its carbon skeleton from that of the heptanes. The similarities among these three antibiotics are stressed in Table 12, in which it can be seen that the polyol region of the nystatin molecule differs from that of candidin only in the oxidation state of the C-7 substituent. Filipin III (XIII) and fungichromin (XIV) are also very closely related, the former being 14-deoxyfungichromin. The Filipin I complex (25, 209) probably consists of isomers of Dideoxyfilipin III, Filipin II is probably C-3 or C-1' Deoxyfilipin III, and Filipin IV is a stereoisomer of Filipin III, again probably at the C-3 or C-1' position (209). Dhar et al. (83) point out that, by making minimal assumptions about stereochemistry, the conformation of the lagosin (fungichromin) molecule is such that the hydroxyl groups are rigidly orientated. Umezawa et al. (288) found that the chromophore (XXIII) of fungichromin is also present in pentamycin (moldcidin B). It has been suggested (29) that pentamycin is identical to

TABLE 12. Homologies between nystatin, candidin, and amphotericin B

No. of carbon atom	Nystatin	Candidin	Amphotericin B
1	=O	=O	=O
2			
3	—OH	—OH	—OH
4			
5	—OH	—OH	—OH
6			
7	—OH	=O	
8			—OH
9			—OH
10	—OH	—OH	
11	—OH	—OH	—OH
12			
13	=O	=O	=O
14			
15	—OH	—OH	—OH
16	—COOH	—COOH	—COOH
17	—OH	—OH	—OH
18			
19	Mycosamine	Mycosamine	Mycosamine

the carbon chain. The eurocidins (XVII and XVIII) are the only representatives of the classical pentanes for which any detailed structural proposals have been made. Mechlinski et al. (182) and Borowski et al. (45) have shown that amphotericin B can exist in the hemiketal form (XXII), in which an oxygen bridge is formed between carbon atoms 13 and 17; Chong and Rickards (66) and Borowski et al. (41) also suggest that this might be the case for nystatin and candidin, respectively. It is clear from an examination of structures VIII to XI that hemiketal formation could also occur in Rimocidin (between carbon atoms 11 and 15), pimaricin, lucensomycin, and the tetrins (between carbon atoms 9 and 13), and also in the zurocidins (XVII and XVIII) if a keto function were located at carbon atom 11 in the latter. Very recent work (66a) has shown that pimaricin and lucensomycin do in fact exist in a hemiketal form. This is thus the second example of an exception to the general rule that the macrolide antibiotics have only one ring structure (the other is the epoxide ring).

The structures for the aglycones of DJ 400 B<sub>1</sub> (XXI) and B<sub>2</sub> (which lacks the diene sidechain at carbon atom 41) are the only examples as yet of assignments in the aromatic group of heptanes. Hattori (126) has suggested a bicyclic structure for trichomycin A, with a main ring consisting of 31 atoms. This is out of agreement with the observed fact that all the polyenes have an even number of atoms in their macrolide ring (Table 11).

### Chemical Modifications

Until recently, all the available evidence (see below) indicated that the structures contained in polyene molecules which confer toxicity may also be responsible for antimycotic activity (148), so that chemically modified compounds with reduced toxicity would be expected to have diminished biological activity as well. Significant chemical modification of this group has been made only with respect to increasing pharmaceutical acceptability, and these compounds, described below, to date have made little if any impact clinically. *N*-acetylation of the amphoteric polyenes converts them into free acids, which are then capable of forming salts which are water soluble (237). Such acetyl derivatives are, however, five to ten times less active than the parent compounds (162), but even so are still more active than native tetranes. In contrast with the results with heptanes, Lechevalier et al. (162) found that acetylated nystatin was virtually devoid of biological

activity. Candidin and amphotericin B form monoacetyl derivatives, whereas candidin and trichomycin form both monoacetyl (still amphoteric) and diacetyl (acidic) derivatives, for the primary amino groups in both mycosamine and the aromatic moiety are available. *N*-acetylcandidin was used by Cirillo et al. (67) in laboratory studies; this compound has also been recommended for use in tissue culture (223), for in comparison with the parent compound, its decreased toxicity was in excess of its diminished biological activity (30-fold as opposed to five- to tenfold, respectively). Perimycin is unusual among the heptanes in lacking a carboxyl group, and because it contains a secondary amino group in its aromatic sidechain, it is a monoacidic base by virtue of the primary aminogroup in the perosamine moiety. *N*-acetylation of the latter would lead to a neutral compound, whereas *N*-succinylation will give an acidic compound analogous to those described above. *N*-succinylperimycin was found by Michalska (185) to have about 12% of the anti-*Candida* activity of the parent compound.

Recently, Schaffner et al. (39, 181, 129) took a new approach to the problem of obtaining water-soluble derivatives of the polyene antibiotics. Esterification with diazomethane of the free carboxyl group of amphotericin B or its *N*-acylated derivatives was found to cause no loss of biological activity. Furthermore, the hydrochlorides of the methyl esters of amphotericin B, nystatin, pimaricin, mediocidin, candidin, and trichomycin were highly water soluble (>20 mg/ml) and retained the full activity of the respective parent compound while being of greatly decreased toxicity. Thus, it seems possible that the toxicity of the polyene antibiotics may be connected in some way with their lack of water solubility and may perhaps be a consequence of their micellar nature in aqueous solvents.

Cocchi and his colleagues (68, 69, 225) solubilized nystatin by making a monohydrochloride. This derivative was reported to be as active as nystatin against *Candida albicans* in vitro and was effective in the treatment of infections with *C. albicans* when it was administered orally or by aerosol in doses of 5 to 39 mg daily.

The fact that certain polyenes (e.g., nystatin and amphotericin A) form complexes with CaCl<sub>2</sub> which are soluble in methanol has greatly assisted in their industrial purification (89, 91).

Virtually all of the chemical interest in the polyene group has been, not unnaturally, devoted to structural studies, and therefore an almost complete gap exists in our knowledge

about the natural degradation products of these compounds. The liability to light and heat of the polyenes as a group is very well known but rather imprecisely stated. There is no doubt that some polyenes, pimaricin for instance, are more thermostable than others (266). The thermostability of Filipin in the crystalline state has been investigated by Tingstad and Garrett (281), and the aerial oxidation and photolysis of Rimocidin and pimaricin has been studied, in solution, by Dekker and Ark (80). Garrett and Elbe (93, 103, 104) carried out a series of careful kinetic investigations into the thermal breakdown and photolysis of the non-macrolide tetrane fumagillin, both in solution and in the crystalline state; first-order kinetics were usually followed for photolysis, with loss of characteristic absorption peaks, but thermal degradation of this antibiotic and of Filipin was more complex kinetically. Thermal inactivation of nystatin was associated with loss of the polyene moiety, and its rate was considerably depressed in the presence of the antioxidant gallate (21). More recently, Rickards et al. (228) have investigated the autoxidation of methanolic solutions of lagosin and Filipin, and they concluded that the reaction products were tetranic 16 and 17 epoxides. Lagosin was much more resistant to aerial autoxidation than was the Filipin complex, and the presence of an antioxidant considerably prolonged the active life of these compounds, as well as that of nystatin (324). From a practical point of view, it is unsatisfactory that no detailed data are available on the photostability of amphotericin B. This compound, when being administered intravenously, must of necessity be exposed to the light in solution for a period of some hours. The glass of the bottle will shield this solution from light with wavelengths of less than about 350 nm.; but the most damaging radiations will be those with wavelengths of between 380 and 410 nm (i.e., blue light), the wavelengths at which amphotericin B shows its absorption maxima (298). The use of an amber bottle, or, better still, a light-proof cover will cut out these harmful radiations, but it would be desirable to know exactly to what extent the biological activity of amphotericin B solution is affected by exposure under various conditions to white light at room temperature. Dekker and Ark (80) suggested that UV photolysis of pimaricin brought about a *trans* to *cis* isomerization; aerial oxidation and photolysis could be inhibited by chlorophyll. The same conclusion was also reached by Zondag et al. (325), who observed that the photolysis of pimaricin by visible light occurred in the presence of ribo-

flavin, and by Siewert and Kieslich (254). It should be noted that there is not always a correlation between loss of biological activity and loss of UV absorption (125a).

## BIOLOGICAL ASPECTS

### Natural Occurrence

Many polyenes have been described since the first report of nystatin (then called fungicidin) by Hazen and Brown (127) in 1950. Fradycin was reported in the same year (269), Rimocidin (79) and endomycin (116) in 1951, and ascocin, chromin, mycelin, trichomycin, and antimycoin A in 1952 (303). The discovery rate of polyenes can be charted with reference to the periodic reviews of this subject; thus, in chronological order, Dutcher (89) listed 16 polyenes and Vining (303) listed 41; Waksman et al. (312) said that "nearly 50" such compounds have been reported, and Dutcher (90) stated that "sixty or seventy" polyenes have been isolated. Oroshnik and Mebane (204) listed 57 compounds, for some of which documentation is extremely scanty. Eighty-seven compounds, most of which have reasonable chemical documentation, are listed in tables 2 to 9; some compounds have been omitted because of lack of adequate data. Thus, it is clear that the pace of polyene discovery continues unabated; there is no reason to doubt that there are more polyene antibiotics to be discovered, and some discoveries already made have yet to be published.

With the exception of proticin, all of the polyenes listed in Tables 2 to 9 are produced by members of the *Streptomycetaceae*, namely *Streptomyces*, *Streptoverticillium*, and *Chainia*. The majority come from *Streptomyces* species; Preobrazhenskaya (209) stated that polyenes are most often produced by members of the "griseus" and "aureus" series. For a substantial minority (20 out of 77, 26%) of the antibiotics produced by *Streptomyces* species, however, the producing strain has not been speciated, and there are grounds (255) for believing that the precise identity even of those strains given a specific epithet is open to question. Hence, the possibility that polyene production may be of taxonomic value cannot be assessed until there has been a reappraisal of the whole system of classifying the *Actinomycetales*. Lechevalier et al. (163) are of the opinion that the production of an antibiotic is even less useful in the speciation of the *Actinomycetes* than is pigment production.

Of the 55 different named species of *Streptomyces* appearing in Tables 2 to 9, 32 appear in the list given by Waksman (309) of

well-defined species. The distribution of these strains among Waksman's four main groups, together with the overall distribution of all the 252 species described by him, is shown in Table 13. The higher incidence of polyene-producing strains in group B II (verticil- and melanin-positive) is significant ( $P < 0.001$  by  $\chi^2$  test), whereas in the other groups the observed incidences are not significantly different from those expected.

It appears that, of all the antibiotics produced by the genus *Streptomyces* (311), the polyenes are the most commonly occurring single group (217). The results of three surveys of polyene production by *Streptomyces* species (1, 217, 299) indicate that from 34.2 to 8.8% of strains isolated from various soil sources produce polyenes. As can be seen in Table 14, Vanek et al. (299) and Pledger and Lechevalier (217) found heptanes in great predominance, whereas Aburatami et al. (1) and Ball et al. (19) found more equal numbers of heptanes and tetranes. The reasons for the obvious discrepancies are not entirely clear, but it seems that the samples examined by Ball et al. (19) came from the widest variety of sources.

#### Co-production of Antibiotics by Polyene-Producing Strains

There are numerous instances of organisms producing more than one polyene antibiotic; as noted earlier, this situation has given rise to great difficulties, on occasion, in separating the different polyenic entities and in the establishment of precise molecular formulas, particularly before the widespread use of mass spectrography. More often than not, polyenes co-produced in this way have been shown to be closely related chemically (tetrins A and B, the Filipin complex, mycotocins A and B [the flavofungins], chainin and its neo- and nor-analogues, and eurocidins A and B, for example), differing by not more than a methyl or a hydroxyl group. The 12 *Streptomyces surinam* heptanes, nystatins A<sub>1</sub> and A<sub>2</sub>, and the "can-

TABLE 13. Distribution of polyene-producing strains of *Streptomyces* among the four groups described by Waksman (309)

Incidence of strains		
Waksman group	All described by Waksman	Polyene producers
A I	152/252 (60.5%)	16/32 (50%)
A II	69/252 (27.4%)	6/32 (18.8%)
B I	8/252 (3.2%)	2/32 (6.3%)
B II	23/252 (9.1%)	9/32 (28.2%)

TABLE 14. Incidence of various classes of polyenes produced by *Streptomyces* spp. isolated from soil samples

Survey carried out by	Total no. of polyenes examined	Heptanes (%)	Hexanes (%)	Pentanes (%)	Tetranes (%)
Pledger and Lechevalier (217)	28	93	0	7	0
Ball, Bessell, and Mortimer (19)	64	23.4	1.5	36	39.1
Vanek et al. (299)	203	89.2	1.48	5.42	3.9
Aburatami et al. (1)	88	56.9	0	6.4	34

didin complex" (candidin, candidoin, and candidin) also appear to be closely related chemically. There is insufficient evidence to allow the same conclusion to be drawn for the methylpentanes moldcidin A and pentamycin (moldcidin B), but it is worth pointing out that if a mycosaminyl residue ( $C_6H_{12}NO_4$ ) is added to the molecule of pentamycin, the resulting formula closely approximates that given for moldcidin A.

In the cases mentioned in the preceding paragraph, co-production of polyenes involves production of two or more compounds with the same number of double bonds; this is not always the case, however. The tetranes amphotericin A and endomycin A are co-produced with the heptanes amphotericin B and endomycin B, respectively. Of these four compounds, the structure of only amphotericin B is known; however, because the amphotericins are of virtually identical molecular weight, it is not impossible that their chemical structures may be similar, notwithstanding differences in degree of unsaturation (cf., nystatin and candidin; Table 11). Because information about the endomycins is almost totally absent, speculation about possible similarities in structure is in this case impossible.

However, from data presented in this review, it can be calculated that the molecular weight of endomycin A is about 845 and that for endomycin B (see Table 7 for  $E^{1*}$  and interpolation from data in Table 10 for estimate of  $E_m$ ) about 800, so these two compounds are at least of similar molecular weight. Thus, there has nowhere emerged from the literature firm evidence that co-produced polyenes are of dissimilar structure, and there are certain indications, as referred to above, that such co-produced compounds may all be chemically closely interrelated.

In addition to co-production of polyene com-

pounds, there are numerous examples of production of nonpolyenic antibiotics by polyene-producing organisms. No purpose would be achieved by the mere listing of these compounds, but certain compounds of especial interest and importance are worth mentioning, if only to illustrate the extreme biosynthetic versatility of the *Streptomycetaceae*. Among the compounds showing antibacterial activity, there are three members of the aminoglycoside group, paromomycin (*S. rimosus*), neomycin (*S. fradiae*), and streptomycin (*S. griseus*), and tetracyclines (*S. rimosus* and *S. aureofaciens*) and streptothricin (*S. flavus*). Two of the antifungal compounds are cycloheximide (*S. noursei* and *S. griseus*), which is a glutarimide related to the cytotoxic streptovitacins and which is known commercially as Actidione, and scopafungin (*S. hygrosopicus* var. *enhygrus*). Among compounds showing antiviral activity are distamycin A (*S. distallicus*; 64) and abikoviromycin (co-produced with trichomycin; 283). Cytostatic activity is shown by actinomycin (*S. cellulosa*) and by E-73 (*S. albulus*; 224).

### Biological Activity

**Qualitative activity.** The polyene antibiotics are, generally speaking, virtually without activity against the *Schizomycetes* and exert various degrees of inhibition against many mycopathological species, such as yeasts and dimorphic fungi (e.g., *Candida*, *Cryptococcus*, *Histoplasma*, *Blastomyces*, and *Coccidioides*), dermatophytes (e.g., *Trichophyton*, *Microsporum*, and *Epidermophyton*), and molds (e.g., *Aspergillus* and *Penicillium*). Certain polyenes, however, like resistaphylin and proticin and, to a lesser extent, cryptocidin and dermostatin, for instance, show significant activity against bacteria. It is interesting to note that the nonmacrolide polyenes variotin and fumagillin both have spectra of activity which differ from those of the macrolide polyene antibiotics; variotin is inactive against yeasts and bacteria, but is active against dermatophytes and molds, whereas fumagillin is active only against bacteriophage and amoebae. In addition to their antimycotic activities, many polyenes also inhibit protozoa of medical importance, such as trichomonads, *Entamoeba histolytica*, trypanosomes, and *Leishmania*, as well as pathogenic members of the *Mycoplasmatales*.

**Quantitative activity.** As a general rule intrinsic biological activity in the polyene group increases with the number of conjugated double bonds. Accurate figures to support this widely made assertion are difficult to come by, for

experimentally determined minimum inhibitory concentrations (MIC) vary so much between laboratories that comparing data supplied from different sources gives little concrete information. Among factors which may affect the MIC are: inoculum size, temperature, and duration of incubation period and medium composition. Hoepflich and Finn (133a) have investigated the latter factor in some detail; they recommend the use of a completely defined medium, which lacks any potentially inhibitory additives and which is strongly buffered, for the sensitivity testing of yeasts. Only if strictly standardized conditions with a medium whose constitution and properties are fully defined, are adhered to will MIC data from different centers be comparable.

Utahara et al. (243) were among the first to show that tetranes and a pentane (eurocidin) had about one-quarter of the activity, on a weight-for-weight basis, of a hexane (mediocidin) and heptanes against *C. albicans*. Table 15 is based on MIC data obtained by Athar (17) for clinically isolated strains of *C. albicans*; the heptanes are more than one order of magnitude more active, on a molar basis, than Filipin and pimaricin, whereas nystatin, by virtue of its high molecular weight, is about twice as active as the latter two compounds. Although there is little comparative data available for hexane and triene compounds, dermostatin (a hexane) has a MIC for *C. albicans* of 1.4 to 2.8  $\mu\text{M}$  (113), and because cryptocidin (233) is of similar activity, it appears that hexanes are, as would be predicted, intermediate to heptanes and pentanes. Mycotrienin has a MIC of about 8  $\mu\text{M}$  (72) and MM-8 has been stated (13) to be "less active" than nystatin against *C. albicans*; therefore it appears that the trienes are less active than the tetranes. It also has been observed by some workers that heptanes containing an aromatic moiety (e.g., candicidin) are more active than those without (e.g., amphotericin B) (90, 211).

Maniar and Mavdikar (175, 176) reported that the presence of serum enhanced the activity of hamycin, aureofungin, and PA 150, whereas that of trichomycin, lucensomycin, amphotericin B, nystatin, candicidin, candidin, pimaricin, and Filipin was reduced; a similar finding was made by Srivastava et al. (263). Maniar (174) found that serum albumin was responsible for this phenomenon. Pansy et al. (210), however, were unable to confirm by growth inhibition studies the apparent enhancing action of serum on heptanes and suggested it might be an artifact caused by facilitated diffusion or some similar effect. Plate diffusion

assays of the polyene antibiotics (which, being of high molecular weight and hydrophobic, diffuse poorly) are by no means straightforward. Horvath and Koczka (135) reported that increasing the concentration of  $K^+$  increased the activity of nystatin, apparently by reducing the binding of the antibiotic to the cell; these results are difficult to reconcile with those of Marini et al. (178) which indicated that  $K^+$  can in fact reverse some of the effects of nystatin. A possible role for divalent cations is discussed by Lampen (155).

The activity of nystatin is quoted in units; the pure compound contains over 5,500 U/mg. The international standard for nystatin (166) contains 3,000 U/mg. Standard recommended procedures for the microbiological assay of amphotericin B, candidin, nystatin, and pimarin have been published recently (15); an assay method for determining serum levels of hamycin and amphotericin B has also been described (248).

### Uses

Only a few of the polyenes have come into significant clinical use; nystatin and amphotericin B are by far the most widely employed for therapeutic purposes. In any discussion of the medical use of the polyenes, the first point to be stressed is that these are potentially toxic compounds; toxicity is considered in detail in the next section.

The recommended dosage of nystatin is 500,000 U three times daily (i.e., 375 mg per day) by mouth; such treatment, under normal circumstances, rapidly clears candidosis of the alimentary tract. Because the antibiotic is little, if at all, absorbed from the gut, oral nystatin therapy is generally ineffective in the treatment of systemic mycotic infections, although amelioration of some conditions has been reported, presumably because of eradication of primary foci in the intestine (88). Nystatin is also used topically to treat localized infections. Parenteral administration of nystatin is not recommended because of its high toxicity by this route. Amphotericin B is the least toxic of the polyene antibiotics by the intravenous route.

Deep-seated systemic mycoses are treated with amphotericin B, and successful results have been obtained in histoplasmosis, coccidioidomycosis, blastomycosis, cryptococcal meningitis, and disseminated candidosis. The route of administration may be oral, intravenous, or intrathecal, depending on the circumstances; the intravenous dosage of amphotericin B is 0.1 to 1 mg per kg per day, i.e., about 8 to 80 mg daily for an average man. The antibiotic is

routinely infused over a period of about 5 h, but Fields, Bates, and Abernathy (97, 98) have recently found that blood levels can be significantly increased by a more rapid infusion rate; a mean level of 2  $\mu\text{g/ml}$  was attained in this way 1 h after the end of infusion. There are, however, often toxic manifestations after intravenous therapy with amphotericin B, particularly kidney damage, which may be irreversible (296). A maximum total dosage of 3 g of intravenous amphotericin B has been recommended (7). The form of the antibiotic which has been solubilized with deoxycholate (Fungizone) gives higher blood levels, but seems to be more toxic. Generally speaking, it is considered that intravenous therapy with amphotericin B should be given only in cases of absolute necessity, such as life-threatening infections (105).

Orally, much larger doses of amphotericin B may be given, up to 16 g daily, but blood levels obtained under these circumstances are low and variable (88, 89). Oral therapy with amphotericin B gives rise to low and variable blood levels (88) and, although such treatment has been reported to clear lesions in blastomycosis, coccidioidomycosis, and cryptococcosis (259a) and also to prevent relapse of the first two conditions (318a), virtually all administration of amphotericin B is parenteral.

Dutcher (90) gave the annual production in U.S.A. of nystatin and amphotericin B as 100,000 and 20,000 lb, respectively (45 and 9 metric tons, respectively). Certain other polyenes, notably pimarin, hamycin, trichomycin, and candidin have been used to a limited extent, topically or by inhalation, for the treatment of localized mycotic infections. A colloidal preparation of hamycin (23) was found to have some effect on systemic infections in vivo when given orally, and a micronized preparation of the same antibiotic was found to give better blood levels than did pressed tablets (297). The fact that certain polyenes combine activity against yeasts and *Trichomonas* makes them particularly attractive antibiotics for use in vaginitis. Brummer et al. (51) have recently published a succinct guide to the treatment of fungal infections.

Among the polyene preparations available in Great Britain at the beginning of 1972 (191) were nystatin (Nitacin, Nystan, Lederstatin, Siltetrin, Mysteclin, and Bristrex, the last four of which also contain tetracyclines, a practice which has been disapproved of [183]), amphotericin B (Fungilin and Fungizone), candidin (Candeptin), and pimarin (Pimafucin). These are listed for use in the treatment of infections by the oral and parental routes or by



local application (e.g., to the vagina, skin, or oropharyngeal regions).

Other uses of the polyenes are as food preservatives (to prevent the growth of molds, especially on the surface of fruit), for selection of nutritional mutants of yeasts (187, 256, 280), and in tissue culture media, where amphotericin B is widely used in conjunction with benzylpenicillin and streptomycin to suppress the growth of microorganisms.

### Toxicity and Side Effects

In man, infusion of amphotericin B is commonly accompanied by one or more of the following side effects: nausea, vomiting, fever, local thrombophlebitis, anemia, hypokalemia, and increase in blood urea levels. The immediate reactions (nausea, vomiting, and fever) can be controlled to some extent by antipyretics, antihistamines, and hydrocortisone. These effects have been discussed by Butler (55) and by Hill (133). Kidney damage in patients was almost invariable and of long-term duration, if not permanent. The experiments of Gouge and Andriole (121) suggest that renal damage may be to some extent prevented by alkali treatment. A considerable amount of research has been done on the effects of polyene antibiotics on whole cells of various types; they act on mycoplasmas and cells of yeasts, plants, amphibia, and reptiles by causing a loss of intracellular material, probably by damaging permeability barriers (29, 155). The lytic effect on human red-blood cells (29, 145) is of special interest in relation to the clinically observed side effect of anemia (see above). However, there now seems to be little doubt that this normochromic, normocytic anemia is not due to a direct lytic effect *in vivo*, but is caused by a decreased production of red-blood cells brought about by inhibition of bone marrow activity (47, 57).

It is important to note that there is no correlation between the antimycotic and the lytic efficiencies of a polyene antibiotic; the smaller polyenes (e.g., Filipin) are more lytic towards both erythrocytes and bacterial protoplasts than are the large polyenes (e.g., nystatin) (145, 156), whereas the reverse tends to be true for antimycotic activity (see above).

In Table 15 are collected some selected data on the toxicity by various routes of polyene antibiotics, expressed as the mean lethal dose for mice. Figures have been given only where data have been expressed by at least two routes for one antibiotic. It is doubtful whether all the preparations used were pure, so comparisons

between antibiotics are of dubious value; however, it is instructive to compare toxicities of a single compound by the different routes. It is clear that it is by the oral route that the polyenes are best tolerated, followed by the subcutaneous and the intraperitoneal routes in that order, with the intravenous route being that by which the polyenes are most toxic. In two cases, simultaneous figures have been published which enable the chemotherapeutic indexes for candicidin (306) and ayfacticin (142), for the treatment of *C. albicans* infections in mice by the intraperitoneal route, to be calculated; the indexes are 63 to 87, and 100, respectively.

Two more interesting side effects have been reported by Gordon and Schaffner (114, 238). These workers found that the enlarged prostate glands of old dogs were dramatically reduced in size after the oral administration of candicidin, Filipin, or amphotericin B. It was also shown that feeding these compounds caused considerable reductions in serum cholesterol levels. It was assumed that these actions were connected (156) and that they were caused by the sequestration of free cholesterol by combination with polyene molecules in the gut.

Wasilewski (314, 315) and Coskey (73) have recently reported four cases of allergic contact dermatitis due to nystatin.

### Resistance

Yeasts are intrinsically capable of giving rise to polyene-resistant variants, as work cited below has shown, so it perhaps is surprising that, after some 15 years of antibiotic use, neither primary nor acquired resistance has, as yet, presented a problem in the use of these compounds. In numerous training experiments, in which *Candida* strains were grown *in vitro* by serial subculture in the presence of gradually increasing concentrations of polyene antibiotics, it appeared that only some strains possessed the ability to express resistance. Thus, Donovick et al. (85) and Littman et al. (169) failed to obtain polyene-resistant strains of *C. albicans*, and Athar and Winner (18) succeeded in training fewer than half of the strains tested to polyene-resistance. Multistep variants of many strains of several species of *Candida* have been made in training experiments by using various polyenes (46, 128, 130, 169, 172, 186, 216, 262, 265). Single-step variants have been found to occur naturally in one *C. albicans* strain at a frequency of about  $10^{-7}$  (213), have been made in other strains by Saltarelli (235) by using UV radiation, and have been found by Hamilton-

TABLE 15. Toxicity of various polyene antibiotics by different routes in mice

Compound	LD <sub>50</sub> in mice (mg/kg)			
	Intravenous	Subcutaneous	Intraperitoneal	Oral
Akitamycin		100	16	150
Aliomycin			45	2,650
Amphotericin B	4-6.6		280	>8,000
Antifungin 4915	3.5	25		2,000
Antimycoin A		>532	204	
Ascocin	12.5	168	8.6	>500
Ayfactin			3.8	>1,000
Candididin		160-280	2.1-7	90-400
Candidin B	0.25	4.9	14	>16.3
Capacidin		7.4	4.2	
Eurotin	30	60	30	
Fungichromin			16.4	2,100
Hamycin	6-9		8.2-18	100-300
Heptane 757		60	5	
Lucensomycin	44.6		37.1	1,263
Nystatin	3	24	8-14	>3,500
Pimaricin	5-10	5,000 <sup>a</sup>	250 <sup>a</sup>	1,500 <sup>a</sup>
Pentamycin			33.3	1,624
Tetrahexin		400	40	
Trichomycin	2.2	17	2.2	300-1,000
7071 RP		37		250

<sup>a</sup> Rats.

Miller (124) by using the mutagenic agent *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. In *Candida*, resistance was found to be associated with changes in the ergosterol content of the cells (18, 125). Resistant strains have been reported to grow more slowly than the parent strains, to be less virulent (perhaps as a result of their diminished growth rate), and to show no cross-resistance to other antifungal agents, such as 5-fluorocytosine, clotrimazole, and pyrrolnitrin (17, 18, 124). Cross-resistance is, as would be expected, observed between the polyene antibiotics. Quantitatively, acquired resistance to amphotericin B tends to reach a much higher level than that to nystatin, and resistance to Filipin is rather low level.

Hejzlar and Vymola (130) reported that a significant proportion of *C. albicans* strains isolated by them in Czechoslovakia were not inhibited by 56 U/ml of nystatin (i.e., between 2 and 5 times greater than the usual MIC), and Bodenhoff (34) has described an apparent case of in vivo acquisition of resistance during treatment. Besides these two reports, which have to be weighed against a mass of evidence suggesting that the sensitivity of yeasts to polyenes has not changed appreciably over the years (17, 18), there is no cause for pessimism over the future usefulness of the polyenes. However, penicillin resistance in the gonococcus has taken more than 20 years of almost imperceptible buildup

to become of notable clinical relevance (105), and only a slight increase in resistance to polyenes would render them virtually useless as chemotherapeutic agents (183); thus, the present situation does not call for complacency. Fortunately, due to their toxicity, nystatin and amphotericin B tend to be used with much greater care and circumspection than are some other antibiotics; this, indeed, may be a reason for the apparent lack of acquired resistance to the polyenes.

The genetics of polyene resistance have been studied in *Saccharomyces cerevisiae* by Woods and his colleagues (3, 189, 321, 322) and by Patel and Johnston (213, 214). The first group showed that nystatin resistance was controlled by three recessive genes and two dominant modifiers, whereas the latter workers claim that at least one resistance gene is dominant. Patel and Johnston (214) suggest that ploidy is directly connected with nystatin resistance, perhaps because of the increased cell size of the polyploid strains. In this respect it is interesting to note that Hamilton-Miller (124) remarked upon the greater cell size of *C. albicans* polyene-resistant mutants. Woods (320), Molzhan and Woods (189), Bard (20), and Starr and Parks (279) have reported alterations in the sterol content of nystatin-resistant *S. cerevisiae* cells, and the latter authors have suggested that zymosterol may have replaced ergosterol.

Woods and Ahmed (321) reported nystatin-dependance in *S. cerevisiae* and found that resistance to amphotericin B (which was up to 200-fold) was capable of being much more pronounced than that to nystatin (60-fold) and Filipin (2.5-fold). A similar situation seems to exist for *Candida* species (124). Capek et al. (58) isolated a nystatin-resistant strain of *Trichophyton mentagrophytes*, and Capek and Simek (58, 60) have reported that dermatophytes (*T. mentagrophytes*, *T. rubrum*, and *Microsporum gypseum*) produce an enzyme which degrades nystatin provided they have been grown in the presence of either nystatin or amphotericin B (0.8 µg/ml). Such an enzyme thus appears to be analogous to the induced  $\beta$ -lactamase (penicillinase) of *Bacillus* species and *Staphylococcus aureus*; intrinsic resistance was also found to be increased in the resistant dermatophytes (60), and the precise mode of resistance remains unresolved. The same authors (61) also observed that, in *M. gypseum* and *T. mentagrophytes*, loss of sensitivity to polyenes was associated with decreased sterol content.

### Biosynthesis

Early workers discovered that yields of certain polyenes in submerged culture could be increased by the addition of a source of fat, *viz.* oleic acid for fungichromin (173) palm oil, maize oil, lard oil, palmitic acid for lagosin, (28), soya bean meal for sistomycin (95) and aureofungin (277), palmitic acid for Filipin (49), and olive or coconut oil for the DJ400 series (254). There have also been reports by Schaffner et al. (240) that mevalonate stimulates the production of polyenes for antimycin A and by Mohan et al. (188) for perimycin. Perlman (215) has summarized much of the available data on the commercial production of polyenes.

Although a considerable amount of work has been done with isotopic tracers on the biosynthesis of the erythromycin group (300), little has been done on the polyene group (32). The structures of all the polyenes so far elucidated are generally consistent with a biosynthetic pathway involving condensation of acetate and propionate units (the "polyketide" pathway), as was found for the erythromycin group. Thus, nystatinolide is assembled from 3 propionate and 16 acetate units (33) and lucensomycinolide from 2 propionate and 12 acetate units (177). One anomaly is the —OH group in amphotericin B (182); the formation of this can be explained by invoking an epoxide mechanism such as that discussed by Rinehart et al. (229) to account for the differing oxidation states at

C-4 of tetrins A and B. Rinehart et al. (229) also cite unpublished work by Schaffner to the effect that the mycosamine moiety of the tetrins is formed directly from glucose, a finding similar to that made by Birch et al. (33) concerning nystatin.

Liu et al. (170, 171) discovered that the aromatic moieties of candidicin and perimycin are formed directly from glucose, via shikimate and *p*-aminobenzoate. The biosynthesis of polyfungin has been shown to resemble that of fatty acids (222), for methylmalonyl Co enzyme A carboxytransferase and phosphoenolpyruvate carboxylase cooperate to polymerize the two- and three-carbon units.

### Mode of Action

As Kinsky (146) pointed out, there was no definite idea of how the polyenes acted until the early 1960's. Since then, however, a very definite picture has been built up; it has been shown (144, 160) that organisms sensitive to polyenes bind these substances, probably to sterols in the cell membrane. Combination of the antibiotic with the cell causes distortion of the membrane and malfunction whereby essential metabolites leak out (267, 327). Other effects, such as inhibition of glycolysis, respiration, and cell death (155) must be regarded as secondary. Metabolic activities in cell-free systems are virtually unaffected by polyenes (101, 110, 244). Bacteria, intact and as protoplasts, do not take up polyenes and are unaffected by the antibiotics; similarly, fungal protoplasts are as sensitive as are whole cells (144, 157, 178, 252). Among the experiments suggesting close links between sterol and the mode of action of polyenes, two series have been especially convincing. Firstly, all organisms susceptible to polyenes contain sterols (e.g., yeasts, algae, protozoa, flatworms, and mammalian cells), and all resistant organisms do not contain sterols; of especial interest is the fact that *Acholeplasma* (*Mycoplasma*) *laidlawii*, which contains cholesterol, is sensitive to polyenes, whereas when grown in the absence of added sterol this organism does not contain cholesterol and is resistant (96, 317). A similar situation has been shown to exist for *Schizosaccharomyces japonicus* (*S. versatilis*), which, when cultured under anaerobic conditions, contains virtually no ergosterol and is resistant (52). Secondly, sterols can protect polyene-sensitive cells from these antibiotics (118, 158, 328).

Evidence is offered in the papers cited above that binding between polyenes and sterols is

indirect; direct evidence based on spectrophotometry data (e.g., 119, 158) is open to the criticism that the addition of sterol may merely decrease the solubility of the micellar polyene in water. Schroeder, Holland, and Bieber (245) have recently produced incontrovertible direct evidence for binding between sterols and Filipin by use of a fluorometric technique involving the measurement of partial quantum efficiencies. It is interesting that binding decreased the fluorescence of Filipin, but increased that of pimarinic (245) and of lucensomycin (78). Norman et al. (198) have investigated the stoichiometry of the Filipin-cholesterol interaction and report that one molecule of the polyene reacts with 1.5 molecules of sterol under the simplest possible conditions and with 2 molecules in a biphasic system. These authors have also investigated the structural requirements necessary for a sterol with Filipin, and they found that stigmaterol reacted the best.

The polyenes with smaller macrolide rings (e.g., Filipin) have been found to cause much greater damage to membranes than do those with larger rings (e.g., nystatin and amphotericin B); the ultimate effect in this respect is the finding (40) that *N*-succinyl perimycin brings about a membrane lesion which results in the loss of  $K^+$  only from the organism. There seems to be a correlation between the ability to damage yeast membranes and to lyse mammalian erythrocytes (29, 67, 145, 155). Kinsky et al. (148) found that the hemolytic activity of Filipin was intimately connected with the integrity of the polyene chromophore. It is of interest to note that members of the *Mycoplasmataceae* (*Mycoplasma gallisepticum*, *M. mycoides* var. *mycoides*, *M. mycoides* var. *capri*, and *A. laidlawii*) bind nystatin almost as well as does *S. cerevisiae*, yet are scarcely inhibited by it (159, 227). Filipin, however, was very active against *M. gallisepticum* (MIC of 0.5  $\mu\text{g}/\text{ml}$  in comparison with 3  $\mu\text{g}/\text{ml}$  for *S. cerevisiae* (159).

The nature of the membrane lesion has been studied extensively with the aid of artificial membranes and electron microscopy (6, 99, 147, 149, 150, 156, 246, 326). In all cases, by using yeasts and animal membranes under physiological and artificial conditions as well as artificial membrane, Filipin has a much more severe effect than the larger polyene antibiotics. This indicates that membrane damage, *per se*, is not primarily responsible for the inhibition of yeast and cell death, for Filipin is considerably less active, mole for mole, than the larger heptanes (Table 16). Medoff et al. (184) observed that

TABLE 16. Relative activities against *Candida albicans* of five polyene antibiotics<sup>a</sup>

Antibiotic	Assumed molecular weight	Median MIC	
		$\mu\text{g}/\text{ml}$	$\mu\text{M}$
Nystatin	920	3	3.3
Pimaricin	666	5	7.5
Filipin	655	5	7.4
Candicidin	1,200	0.5	0.42
Amphotericin B	920	0.5	0.54

<sup>a</sup> Data calculated from figures of Athar (17).

yeast cell membranes damaged by subinhibitory concentrations of amphotericin B were more permeable to rifampin and 5-fluorocytosine.

The action of the polyene antibiotics on the permeability of membranes has been used in studies of membrane function by using the skin (197) and bladder (24, 249) of toads, chicken intestine (2, 319), and mammalian erythrocytes (56) among other organs. The action of polyenes on isolated mammalian hearts is to induce ventricular arrest (14); this has been ascribed to a selective effect on electrolytic movements. It has recently been suggested (190) that lucensomycin and Filipin have a greater affinity for the membrane of malignant cells than for those of normal cells.

### CONCLUDING REMARKS

As will have become apparent, the polyenic macrolides share virtually no properties with the erythromycin group of macrolides, save possibly some common biosynthetic mechanisms. From the medical view point, the polyenes represent a very important group of broad-spectrum antibiotics active against pathogenic species causing common and distressing ailments. Effective control of mycotic infections in this age of immunosuppression is of vital importance and, when properly used, the polyenes adequately fulfill this need. Their toxicity is outweighed by the lack of resistance to them among commonly occurring yeasts and fungi. From the academic point of view, once the three-dimensional structures of more polyenes have been elucidated (presumably by X-ray crystallography), a common pattern will probably emerge from which a much deeper understanding of the nature of the interaction between polyenes and sterols should be possible. Such an understanding will enable us to increase our knowledge of the structure and functions of biological membranes, one of the basic, most fascinating, and most challenging

problems facing biologists today. It is fitting that these advances should stem from close cooperation between medical and physical scientists, which has been a constant feature of the polyene story.

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